Simultaneous sonochemical-enzymatic coating of medical textiles with antibacterial ZnO nanoparticles

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ABSTRACT

The antimicrobial finishing is a must for production of medical textiles, aiming at reducing the bioburden in clinical wards and consequently decreasing the risk of hospital-acquired infections. This work reports for the first time on a simultaneous sonochemical/enzymatic process for durable antibacterial coating of cotton with zinc oxide nanoparticles (ZnO NPs). The novel technology goes beyond the "stepwise" concept we proposed recently for enzymatic pre-activation of the fabrics and subsequent sonochemical nano-coating, and is designed to produce "ready-to-use" antibacterial medical textiles in a single step. A multilayer coating of uniformly dispersed NPs was obtained in the process. The enzymatic treatment provides better adhesion of the ZnO NPs and, as a consequence, enhanced coating stability during exploitation. The NPs-coated cotton fabrics inhibited the growth of the medically relevant *Staphylococcus aureus* and *Escherichia coli* respectively by 67 % and 100 %. The antibacterial efficiency of these textile materials resisted the intensive laundry regimes used in hospitals, though only 33 % of the initially deposited NPs remained firmly fixed onto the fabrics after multiple washings.

KEYWORDS: cellulase, sonochemistry, zinc oxide, nanoparticles, antibacterial coatings, medical textiles

1. Introduction

The use of antibacterial textiles is in the list of preventive measures to reduce the bioburden in clinical settings and consequently diminish the risk of hospital-acquired infections [1,2]. Such materials prevent the transmission of microorganisms, impairing the common transfer routes for pathogen spreading [3–5]. Consequently, the antibacterial treatment became an integrated step in the production of medical textiles, such as wound bandages, hospital bed sheets, surgical uniforms and patients' pajamas [6]. The choice of an antibacterial agent is the key to obtain effective bactericidal or bacteriostatic coatings and depends primarily on the required efficacy towards specific microorganisms. However, a major concern remains the release of the active agents during fabric exploitation, which compromises the durability of the antibacterial effect and the safety at use [5,7]. The routine adoption of antibacterial textiles in clinical practice inevitably calls for facile in terms of application and durable coatings, whereas the market demands attractive manufacturing cost [8].

Inorganic nanoparticles (NPs) are claimed to be more biocidal than many conventional antibiotics, which utilization at high concentrations can induce adverse effects and toxicity to human cells [9,10]. The use of antibacterial NPs is also among the most promising strategies to overcome the microbial drug resistance [11]. The mechanism of NPs antibacterial action involves generation of reactive oxygen species (ROS) followed by the disruption of the bacterial cell membrane [10,12–14]. However, the antibacterial potential of the inorganic NPs, except for the silver NPs, has not been exploited sufficiently for the development of medical textiles.

The production of durable antibacterial textiles embedded with inorganic NPs often requires time-consuming fabric pretreatments such as chemical or plasma activation, in addition to

subsequent coating stabilization using different cross linking techniques [15–17]. Using enzymes as tools for activation of textile surfaces would avoid the use of harsh chemicals and allow to impart new functionalities to the fibrous substrates at mild processing conditions [18].

As an alternative to the existing finishing technologies, a facile one-step sonochemical route has been suggested for uniform deposition of inorganic NPs on the surface of solid substrates, including textiles [19,20]. The formation, growth and collapse of cavitation bubbles, formed upon sonication of liquids, determine the main features of the deposition process. Microjets formed after the bubbles' collapse drive at huge velocities the NPs encountered in their vicinity towards the solid surface where physico-chemical interactions occur during the collision. In a previous work, we combined the sonochemical deposition of NPs with an enzymatic pre-activation of cotton fabrics in order to create anchoring points for embedding the NPs on the textile surface, thereby improving the durability of the coating. In such way, antibacterial fabrics with durable antibacterial effect were obtained in a two-step process comprised of a cellulase pre-treatment followed by a sonochemical coating with ZnO NPs [21]. The particles were generated *in situ* from zinc acetate in water/ethanol alkaline solutions, and deposited on the fabrics using high intensity ultrasound. ZnO NPs were selected due to their general acceptance as biologically safe for humans and the lack of coloration [10,22]. The enzymatic pre-activation of the fabric surface resulted in deposition of smaller particles with enhanced antibacterial activity, improved adherence on the fibers and, consequently, durable antibacterial effect. Despite of these benefits, the industrial acceptance of such two-step process is hampered by the requirements for shorter processing time and simplicity of the operations. Therefore, the challenge was to achieve the above effects using a single step process.

Normally, heterogeneous enzyme catalysis for modification of solid substrates, such as textile fibers, is a time-consuming operation. In particular, the efficiency of hydrolytic enzymes, e.g. cellulases, depends on the mass transfer from the enzyme solution to the solid substrate. Thus, intensifying the mass transfer would shorten the time for fabric activation and lower the amount of the biocatalyst necessary for hydrolysis. Ultrasound (US) has been applied previously as a way to improve the performance of cellulases in de-sizing, scouring, bleaching, mercerization and dyeing of cotton [23–25].

The objective of this work is to combine biocatalysis and physicochemical processing in a single-step, industry-attractive technology for durable coating of medical textiles with antibacterial NPs. The coating consists in embedding of ZnO NPs onto cotton fabrics in a 30 min simultaneous sonochemical/enzymatic process. The US is employed to boost the rate of the enzymatic hydrolysis and create on the cotton surface a larger number of reducing sugar ends for better NPs adhesion and durability of the antibacterial effect. The activity of the fabrics will be evaluated against the clinically relevant bacteria *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) after multiple washing cycles with a non-ionic detergent at hospital laundry regimes (75 °C).

2. Experimental section

2.1. Materials, reagents and bacteria

Bleached 100 % woven cotton fabric (144 g/m^2) was supplied by Davo SRL (Romania). Cellulase formulation Kappacell ETU 39 for biopolishing of cotton textiles (5.86 mg/mL protein, 1.2 mM glucose equivalents released per min, averaged over 60 min, pH optimum 5 - 8) was purchased by Kapp-Chemie GmbH & Co. KG (Germany). ZnO NPs (size <100 nm) dispersion in water, potassium sodium tartrate tetrahydrate and sodium hydroxide and 3,5-dinitrosalicylic acid (DNSA) were purchased from Sigma-Aldrich (Spain). Gram-negative *E. coli* (ATCC 25922) and Gram-positive *S. aureus* (ATCC 25923) were used in the antibacterial activity assays. Plate count agar and all other reagents for bacterial studies were purchased from Sigma-Aldrich unless otherwise specified.

2.2. Cellulase activity measurements

The cellulase activity was measured using the filter paper (FPU) assay [26]. Reactions were carried out in 50 mL test tubes with Whatman No. 1 filter paper strip (1 x 6 cm, 50 mg) in 1.0 mL distilled water and 0.5 mL enzyme formulation. The mixtures were incubated at temperatures ranging from 20 to 65 °C for 1 h. Thereafter, a colorimetric reagent (DNSA) was added to quantify the amount of reducing sugars. The DNSA solution was prepared by dissolving 120 g sodium potassium tartrate in 80 mL of previously heated (60 °C) 0.2 M NaOH, prior to addition of 200 mL of 96 mM DNSA and volume completion to 400 mL with distilled water. The reaction mixtures were placed in a boiling water bath for 5 min, cooled to room temperature and diluted with 20 mL distilled water prior to measuring the absorbance at 540 nm

with Infinite M200 (Tecan, Austria) multiplate reader. All experiments were performed in triplicate.

2.3. Enzyme stability in US field

One mL of cellulase product diluted to 50 mL with water was subjected to US irradiation (20 kHz, 21.5 W, 17.30 W/cm^2, 0.43 W/cm^3 and 35 % of amplitude) for 30 min at temperatures ranging from 20 to 60 °C. After the treatment, enzyme aliquots (0.5 mL) were incubated for 1 h at 55 °C with Whatman No. 1 filter paper strip (1 x 6 cm) and the reducing sugars released were measured using the FPU assay as previously described. The residual enzyme activity was calculated as a percentage of the activity of the enzyme not exposed to US. The possible effect of the sonochemical treatment on the tertiary and secondary structure of cellulase was assessed by measuring the intrinsic fluorescence of the enzyme as a result of protein unfolding and denaturing. For the purpose of the assay, cellulase water solutions not exposed (control) and exposed to US treatment (30 min) were evaluated. The fluorescence was measured at room temperature (25 ± 1 °C) with Quanta Master 4 spectrofluorometer (PTI, USA) at 280 nm excitation wavelength (slit = 2 nm), 300 - 450 nm emission wavelength (slit = 2 nm) and 1200 nm/s of scanning speed.

2.4. Effect of the US parameters on the enzyme performance

To study the hydrolytic potential of the cellulase toward cellulose substrate under sonication, the US amplitude of vibration (and thus intensity) was varied to determine its effect on the yield of the enzyme catalyzed reaction expressed in reducing sugars concentration. For this aim, 1 mL of cellulase solution (diluted to 50 mL with water) was subjected to ultrasonic irradiation at

different US amplitudes (range of 20 – 40 %) for 30 min at 55 °C and in presence of 0.5 g of Whatman No. 1 filter paper as a substrate. Thereafter, the liberated reducing sugars were determined using the aforementioned method (FPU assay). Control treatments without enzyme were carried out in parallel. All results are reported as mean values ± standard deviation (n = 3).

2.5. Ultrasound-enzyme assisted coating of cotton with ZnO NPs

The sonochemical coating was carried out using an ultrasonic transducer Ti-horn (20 kHz, Sonics and Materials VC750, USA). By measuring the time-dependent increasing of the temperature in the ultrasonic glass jacked vessel, the US intensity (17.30 W/cm^2), density (0.43 W/cm^3) and power (21.5 W) used for the textile treatment were calorimetrically determined. The cotton samples (5 x 10 cm, approx. 0.7 g) were immersed in the ultrasonic pot containing 50 mL ZnO NPs aqueous solution (1 mM) and 2 % of weight of fabric (owf) cellulase formulation and the coating of the cotton samples was carried out during 30 min at 55 ± 2 °C and amplitude of 35 %. To maintain the fabric at the bottom of the US pot without using any additional accessories, the fabric sample was cut bigger than the diameter of the pot (4 cm). Thereafter, the sample was folded in a way that its diameter is slightly wider than the diameter of the pot, thereby once placed at the bottom of the pot the contact/friction of its edges with the walls prevents it from moving during the sonochemical process. The sample area exposed to the US irradiation was used for the further experiments. The ultrasonic horn was dippen 1 cm in the ZnO dispersion at a distance from the fabric of approximately 3 cm. Thereafter the samples were thoroughly washed with distilled water to remove the loosely fixed particles. Controls were prepared by sonochemical deposition of ZnO NPs in presence of temperature-denatured enzyme.

2.6. Characterization of the coatings

The amount of ZnO deposited on the fabrics was determined after extraction with 0.5 M nitric acid. Inductive coupled plasma atomic emission spectroscopy (ICP - AES) analysis using ULTIMA JY2501 (France) was carried out to probe the concentration of solubilized Zn^{2+} ions and the obtained results are reported as mean values ± standard deviation (n = 3). The surface morphology of the coatings were studied with high-resolution scanning electron microscope (HRSEM) Quanta 200 FEG from FEI (USA). For both washed and unwashed samples, images were obtained from fabrics treated in three independent experiments. ImageJ software was used to build three size histograms of the ZnO NPs deposited on to the fabrics.

2.7. Antibacterial tests

Antibacterial performance of the coatings was assessed according to the standard shake flask method, recommended by the American Society for Testing and Materials for permanently immobilized active agents on fabrics (ASTM-E2149-01). The method provides quantitative data for measuring the reduction rate in number of colonies formed, converted to the average colony forming units per milliliter of buffer solution in the flask (CFU/mL). A single colony from the corresponding bacterial cultures was used for the preparation of *S. aureus* and *E. coli* suspensions. The culture was then incubated at 37 ºC and 230 rpm and growth overnight in sterile nutrient broth (NB, Sharlab, Spain). The inoculated bacterial culture was diluted until solution absorbance of 0.28 ± 0.01 at 475 nm was reached with sterile buffer (0.3 mM KH_2PO_4), which concurs to 1.5 ÷ 3.0 x 10^8 CFU/mL. This bacterial culture was then diluted appropriately into sterile buffer solution to obtain a final concentration of 1.5 ÷ 3.0 x 105 CFU/mL (working

bacterial dilution). Thereafter, the cotton samples (35 mg) were incubated with 5 mL bacterial suspension (1.5 ÷ 3.0 x 105 CFU/mL) at 37 ºC and 230 rpm for 60 min. The suspensions were serially diluted with 0.3 mM KH_2PO_4 sterile buffer solution, plated on a plate count agar and further incubated at 37 ºC for 24 h to determine the number of bacteria colonies. Antibacterial efficiency is reported as percentage of bacteria reduction calculated as the ratio between the average number of surviving bacteria before (A) and after (B) contact with the coated fabrics using the following formula:

Bacteria reduction (%) == [(A-B)/A] x 100

The durability of the antibacterial effect was assessed after 10 washing cycles in a laboratory machine (Ahiba Nuance, Datacolor) in presence of 0.1 g/L non-ionic surfactant Cotemol NI (Colorcenter, Spain), liquor to good ratio 30 : 1, for 15 min and 30 rpm, at 75 °C as recommended by Walter and Schilinger [27]. The pH of the water used in the washing procedure did not change after the surfactant addition. The latter is important as the ZnO dissolution takes place over a wide pH range [28], however at neutral pH the oxide is stable.

3. Results and discussion

3.1.Cellulase activity at the processing conditions

Cellulases catalyze the hydrolysis of 1,4-β-glucosidic bonds in cellulose substrates. These enzymes are widely used in the textile industry to reduce the surface hairiness and improve the evenness of cotton fabrics in a process called biopolishing. During this process, and as a result of cellulose hydrolysis, novel reducing sugar ends appear on the fibers surface providing anchoring points for further functionalization. The efficiency of the activation process depends on the mass transfer from the processing liquid towards the surface of the textile material, usually intensified by high level of mechanical agitation [29,30]. It was demonstrated that controlled US accelerates this mass transfer without damaging the fibers, and results in better uniformity of the enzymatic treatment over a shorter period of time [24]. Thus, to illustrate the surface effect of the simultaneous US-enzymatic treatment, the morphology of the fibers after 30 min of sonication (amplitude 35 %, intensity 17.30 W/cm^2, 55 °C) alone and in presence of cellulase was observed via HRSEM. The biopolishing effect of the cellulase in terms of removal of the protruding after the US treatment fibrils (Fig. 1a) was confirmed in Fig. 1b.

Thereafter, as a prerequisite for efficient enzymatic activation of the fabric surface, we studied the cellulase activity as a function of the temperature and the enzyme stability in the ultrasonic field. The temperature profile of cellulase activity at temperatures ranging from 20 to 65 ºC showed a maximum at 55 °C (Fig. 2a).

During US irradiation free hydroxyl and hydrogen radicals created by the high localized pressure and temperature could inactivate the enzyme [31]. To verify whether the cellulase activity would be affected by the US applied in the sonochemical coating process, an aqueous cellulase solution without substrate was exposed for 30 min to US irradiation at different

temperatures. Thereafter, filter paper samples were incubated with aliquots of this solution, and the amount of reducing sugars was determined. The residual enzyme activity was calculated as a percentage of the activity of the enzyme not exposed to US irradiation. Only 10 to 20 % activity loss was detected after the US treatment regardless of the temperature in the cell (Fig. 2b), suggesting that the simultaneous sono-enzymatic process would be feasible without compromising significantly the activity of the enzyme.

The hydrolytic potential of the cellulase used in our experiments was studied under ultrasonic irradiation with different amplitudes/intensities. Fig. 3 compares the final reducing sugar concentrations after 30 min of enzymatic hydrolysis on a model cellulose substrate at various amplitudes. The highest amount of reducing sugars released during the enzymatic process was obtained when the US amplitude and intensity were 35 % and 17.30 W/cm^2, respectively. Thus, all further experiments were carried out at these conditions, considered as the optimal for the enzyme performance in presence of US irradiation.

Thereafter, the enzyme activity at the selected processing conditions (amplitude of 35 % and intensity of 17.30 W/cm^2) in terms of reducing sugars released during the 30 min sonication process in the presence of filter paper substrate (0.5 g) was determined and compared to the activity of the enzyme upon mechanical stirring (110 rpm). The assay was performed at the temperature of maximum enzyme activity (55 °C), used further for the enzyme/US coating of cotton with ZnO NPs. A 3-fold increase of the released reducing sugars, and thus, cellulase activity, was observed upon sonication (0.311 ± 0.01 mg/mL) in comparison to mechanical stirring (0.096 ± 0.02 mg/mL), due to the intensified mass transport [24]. In addition, changes in the molecular structure of the enzyme reported by others [32] to explain its increased activity

were not observed in the fluorescence spectra of cellulase before and after sonication (results not shown).

3.2. Simultaneous sonochemical/enzymatic coating of cotton with ZnO NPs

Cotton fabrics were coated with antibacterial ZnO NPs in a simultaneous enzyme/US process carried out with both active and denatured enzyme as a control. ICP - AES measurements were further performed for quantifying the amount of ZnO on the treated fabrics (Table 1). Similar amount of ZnO was deposited sonochemically in the processes using either active or denatured cellulase. In addition to determining the amount of ZnO NPs embedded onto the fabrics, we also examined the durability of the coatings subjected to 10 washing cycles at 75 °C in presence of non-ionic surfactant. The fabrics treated with the active enzyme resisted better the applied washing cycles, though ~67 % of the initially deposited ZnO were removed after the washings. At the same time, 95 % of the ZnO on the fabrics coated in presence of denatured enzyme was washed-off. Greater resistance to washings of the NPs on the samples treated with active enzyme demonstrates the significance of the enzymatic activation of the fibers surface for improved NPs adhesion and more durable treatment. The hydrolytic bio-treatment of the cotton fibers generates free hydroxyl groups on their surface, serving as anchoring points for NPs deposition [21].

The HRSEM surface analysis of the coated samples showed that NPs agglomeration was observed regardless of the enzyme used in the coating process (active or denatured). However, the tendency of the particles to form aggregates was more pronounced for the fabric coated in presence of denatured protein (Fig. 4a, b). More uniformly distributed NPs were observed on the

cotton surface coated in presence of active cellulase (Fig. 4e, f). This was additionally confirmed from the size histograms of the NPs deposited on both fabrics (Fig. 5a, b), showing more narrow distribution of the deposited NPs when the active enzyme was used. These observations corroborate our previous findings that the enzymatic activation of cotton surface leads to uniformly dispersed along the fibers NPs [21]. In the current study a similar effect was achieved using a single-step sono-enzymatic process. The morphology of the fabrics treated in presence of denatured and active cellulase after subjecting to 10 washing cycles is shown in Fig 4c, d and Fig. 4g, h, respectively. On the surface of the fabric coated in presence of denatured enzyme few NPs were found after the washings, while still a dense NPs layer is observed on to the surface of the enzyme treated samples. These observations were in a good agreement with the ICP findings (Table 1), namely NPs improved stability when deposited in a simultaneous sono-enzymatic process.

3.3.Antibacterial activity

ZnO NPs are largely reported as efficient antibacterial agents against both Gram-positive and Gram-negative bacteria. The antibacterial efficiency of the fabrics coated with ZnO NPs was evaluated against *S. aureus* and *E. coli.* The non-washed fabrics treated with active or denatured cellulase showed comparable antibacterial performances (~70 % reduction in bacteria viability) toward *S. aureus* (Fig. 6a). However, after 10 washings the coatings obtained in presence of denatured enzyme lost entirely their antibacterial efficacy toward this bacterial strain. On the other hand, the coating deposited in presence of the active enzyme retained ~50 % of its initial

antibacterial activity, which is in good agreement with the amount of remaining ZnO on this fabric.

All non-washed fabrics were more efficient against *E. coli*, and the samples obtained in presence of active or denatured protein reduced the bacterial viability by 98 % and 90 %, respectively. After washing, however, the antibacterial performance of the coating obtained in presence of active cellulase was maintained. The sample coated in presence of denatured enzyme lost more than 30 % of its initial antibacterial efficiency (Fig. 6b), though the ZnO NPs amount for this sample was reduced by 95 % after the washings. A possible explanation for these results could be that the amount of the ZnO NPs needed to inhibit the *E. coli* growth is much lower than the one initially deposited on to the fabric. On the other hand, the ZnO concentration on the fabrics after the washings is considerably reduced and it is below the concentration needed for full kill of this bacterium but still enough to inhibit 50 % of its growth. These findings reconfirmed that the cellulase treatment improved the durability of the coating effect. The higher resistance of *S. aureus* to ZnO NPs in comparison with *E. coli* is due to the thicker peptidoglycan layer in the cell wall of Gram-positive bacteria, which makes them less susceptible to metal oxide NPs [33, 34]. The results also showed that the durability of the antibacterial effect of the ZnO NP coating obtained in the one step sono-enzymatic coating approach against *E. coli* was higher compared to the already reported durability of the coating obtained in the two step-process (consisting of enzymatic pre-treatment of the cotton textiles followed by sonochemical deposition of ZnO NPs) [21].

4. Conclusions

Novel industry-attractive technologies are required for the manufacturing of antibacterial medical textiles used to reduce the risk of hospital-acquired infections. In this study we assessed the feasibility of a single-step sono-enzymatic process for durable coating of cotton fabrics with antibacterial ZnO NPs. The coating procedure resulted in the uniform multilayer deposition of ZnO NPs onto the fibers. As a consequence of the boosted cellulase activation of the fabric surface during sonication, the adhesion of the coated NPs was improved and thus, the antibacterial performance was ensured. Although the outer NP layers were not firmly fixed on the textiles, 33 % of the initially deposited ZnO NPs remained on the surface after multiple intensive washing cycles. The remaining after washings NPs still exhibited nearly 100 % reduction of the viability of *E. coli*, whereas the efficiency toward *S. aureus* decreased by 50 %. In summary, the one-step sono-enzymatic process is an attractive alternative to the currently used technologies for antibacterial textile functionalization that rely on aggressive and time-consuming chemical pre-treatments to achieve durable antimicrobial fabrics.

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Table 1. Amount of ZnO deposited on the cotton fabrics in a simultaneous sono-enzymatic process (experimental error ± 10 %).

Sample	ZnO, % wt (per 100 g of fabric) after coating		ZnO on the fabrics after 10 washing cycles (%)
	Non-washed	Washed	
Enzyme	0.8048	0.2690	33.4
Denatured enzyme	0.8533	0.0428	5.0

Table and Figure captions

Table 1. Amount of ZnO deposited on the cotton fabrics in a simultaneous sono-enzymatic process (experimental error ± 10 %).

Fig. 1. HRSEM images of cotton fibers after 30 min of sonication (amplitude 35 %, intensity 17.30 W/cm^2, 55 °C) alone (a) and in presence of cellulase (b).

Fig. 2. Temperature profile of cellulase (Kappacell ETU 39) activity (a) and the residual cellulase activity after 30 min of US irradiation (amplitude 35 %, intensity 17.30 W/cm^2) as a function of temperature (b). The residual activity was calculated as a percentage of the activity of

the enzyme not exposed to US at 55 °C for 1 h in presence of Whatman No. 1 filter paper as a substrate.

Fig. 3. Reducing sugars released under different US amplitudes after 30 min of US irradiation at 55 °C in presence of Whatman No. 1 filter paper as a substrate. The US amplitudes of 20, 25, 30, 35 and 40 % correspond to the US intensities of 6.90, 10.40, 13.10, 17.30 and 30.80 W/cm^2, respectively.

Fig. 4. Representative HRSEM micrographs of cotton fabrics coated with 1 mM of ZnO NPs at 55 °C in presence of denatured enzyme before (a, b) and after 10 washing cycles (c, d) and in presence of cellulase before (e, f) and after10 washing cycles (g, h). The images on the left were taken with 15000x, whereas the right-side images were taken with 50000x magnification.

Fig. 5. Size histograms (fitted by Gaussian curves (solid line)) of the ZnO NPs deposited on the fabric in presence of denatured enzyme (a) and cellulase (b) associated with Fig. 3b and 3f, respectively.

Fig. 6. Antibacterial activity of the fabrics coated with 1 mM of ZnO NPs: light gray bars represent non-washed fabrics, whereas dark gray bars show the antibacterial activity of the fabrics after 10 washing cycles at 75°C. The assays were performed with *S. aureus* (a) and *E. coli* (b), after 1 h of contact with the fabrics.

Fig.1

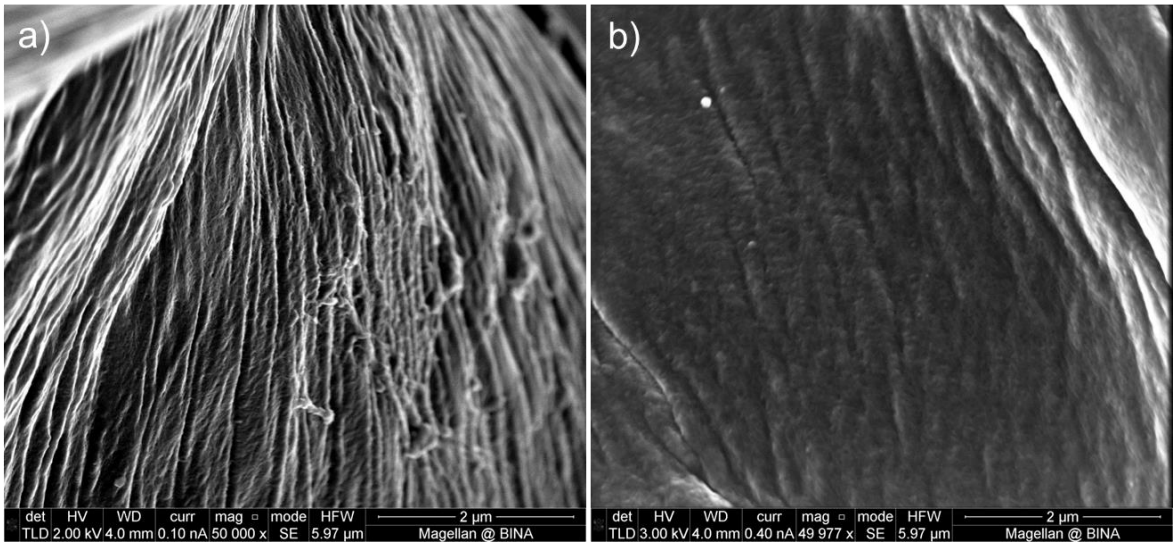

a)
b)
det HV WD curr mag mode HFW 2 µm
TLD 2.00 kV 4.0 mm 0.10 nA 50 000 x SE 5.97 µm Magellan @ BINA
det HV WD curr mag mode HFW 2 µm
TLD 3.00 kV 4.0 mm 0.40 nA 49 977 x SE 5.97 µm Magellan @ BINA

Fig. 2a

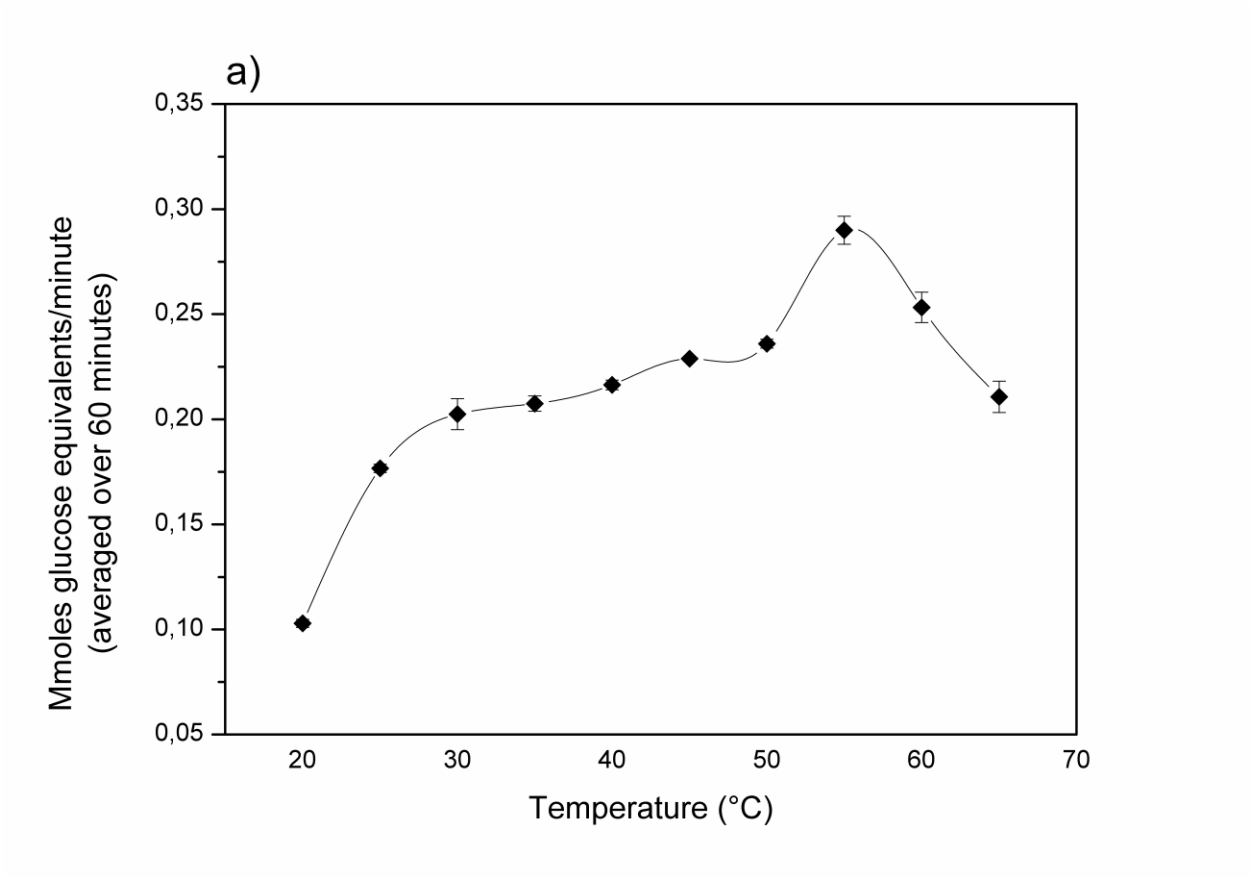

a)
Mmoles glucose equivalents/minute
(averaged over 60 minutes)
0,35
0,30
0,25
0,20
0,15
0,10
0,05
20
30
40
50
60
70
Temperature (°C)

Fig. 2b

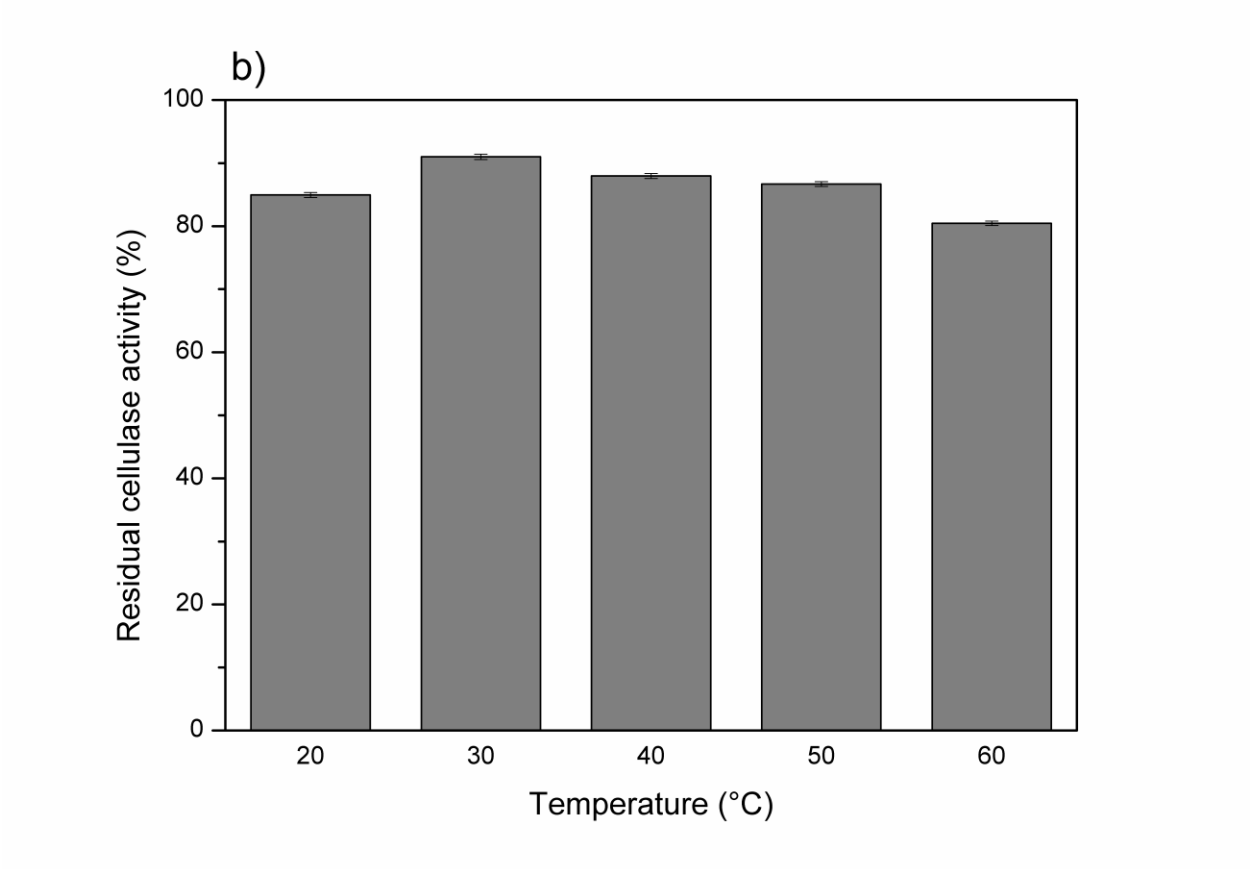

b)
Residual cellulase activity (%)
100
80
60
40
20
0
20
30
40
50
60
Temperature (°C)

Fig. 3

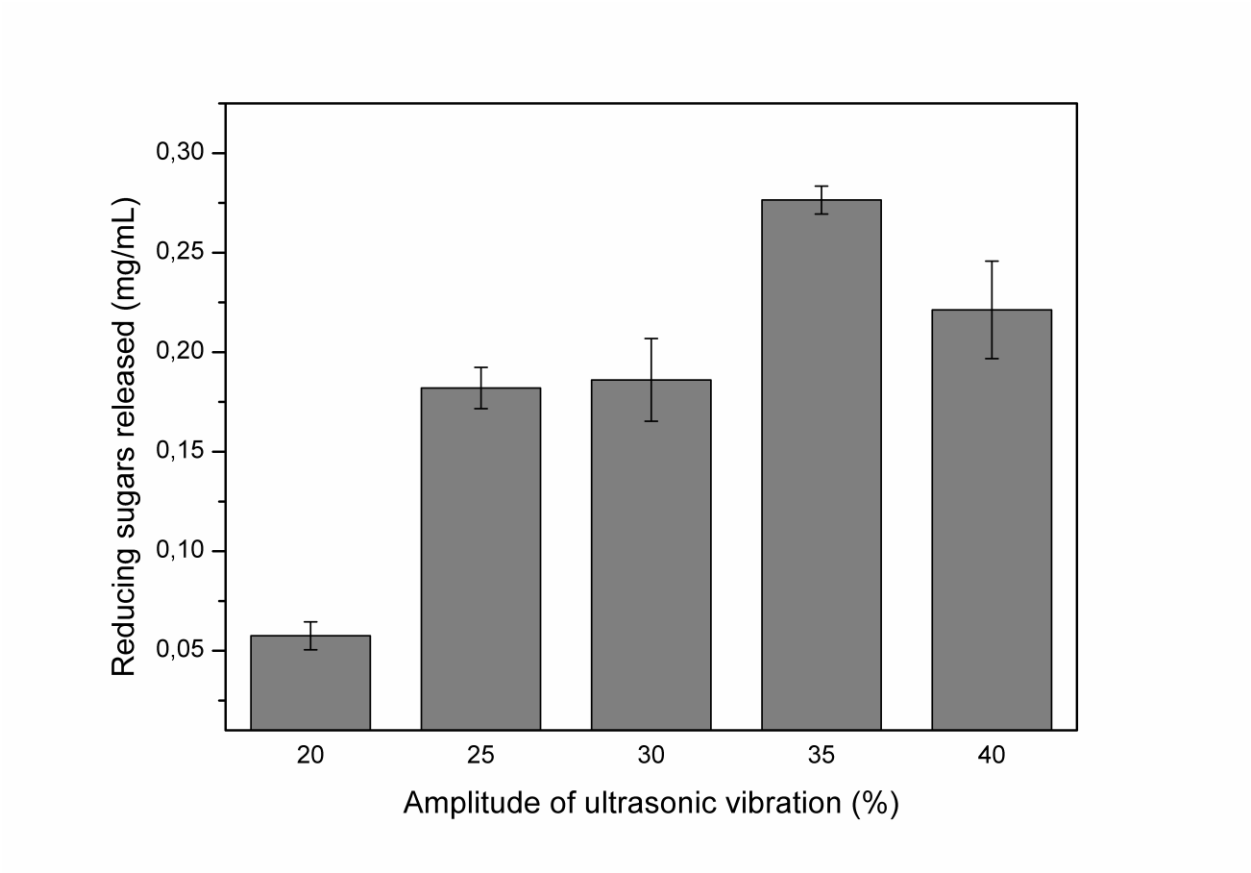

Reducing sugars released (mg/mL)
0,30
0,25
0,20
0,15
0,10
0,05
20
25
30
35
40
Amplitude of ultrasonic vibration (%)

Fig. 4

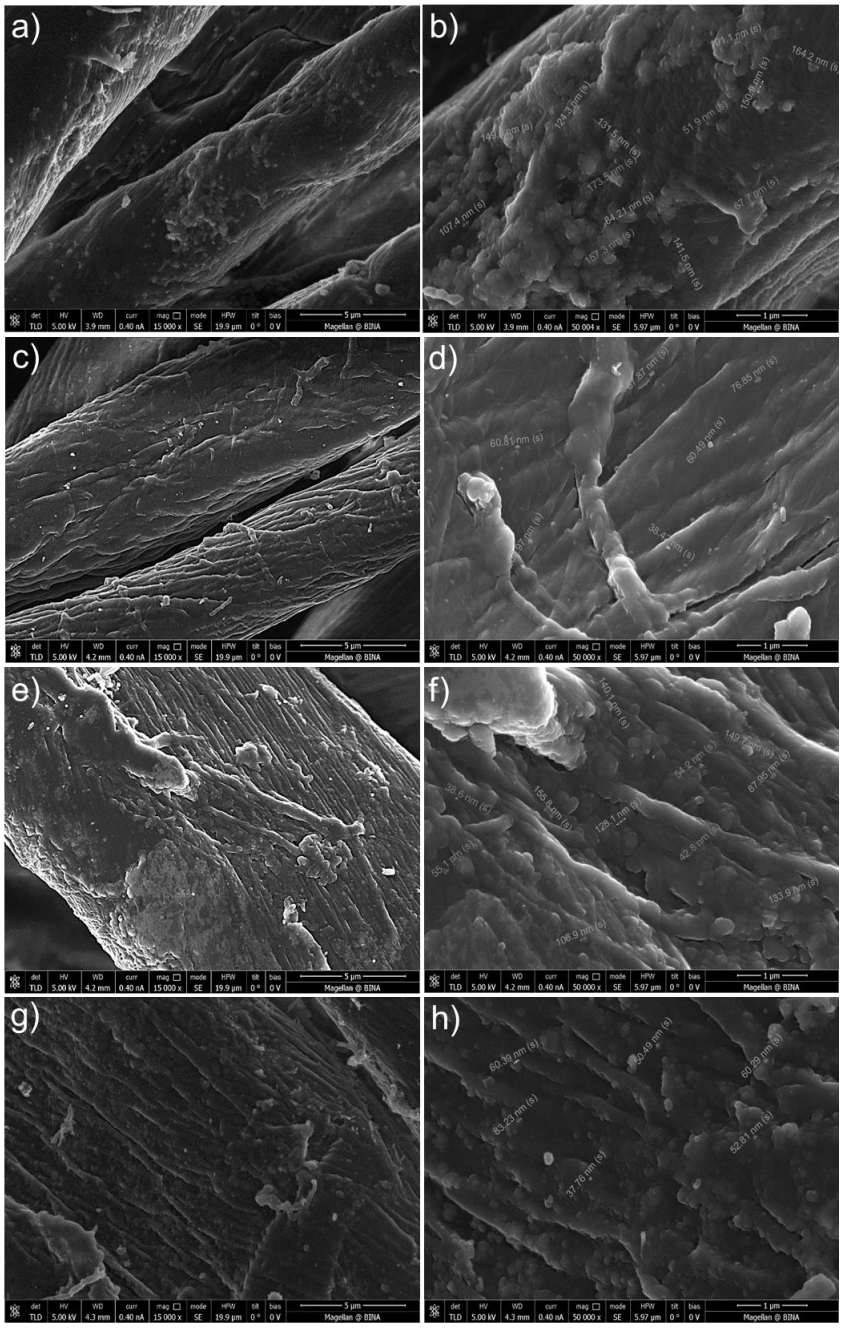


Fig. 5a

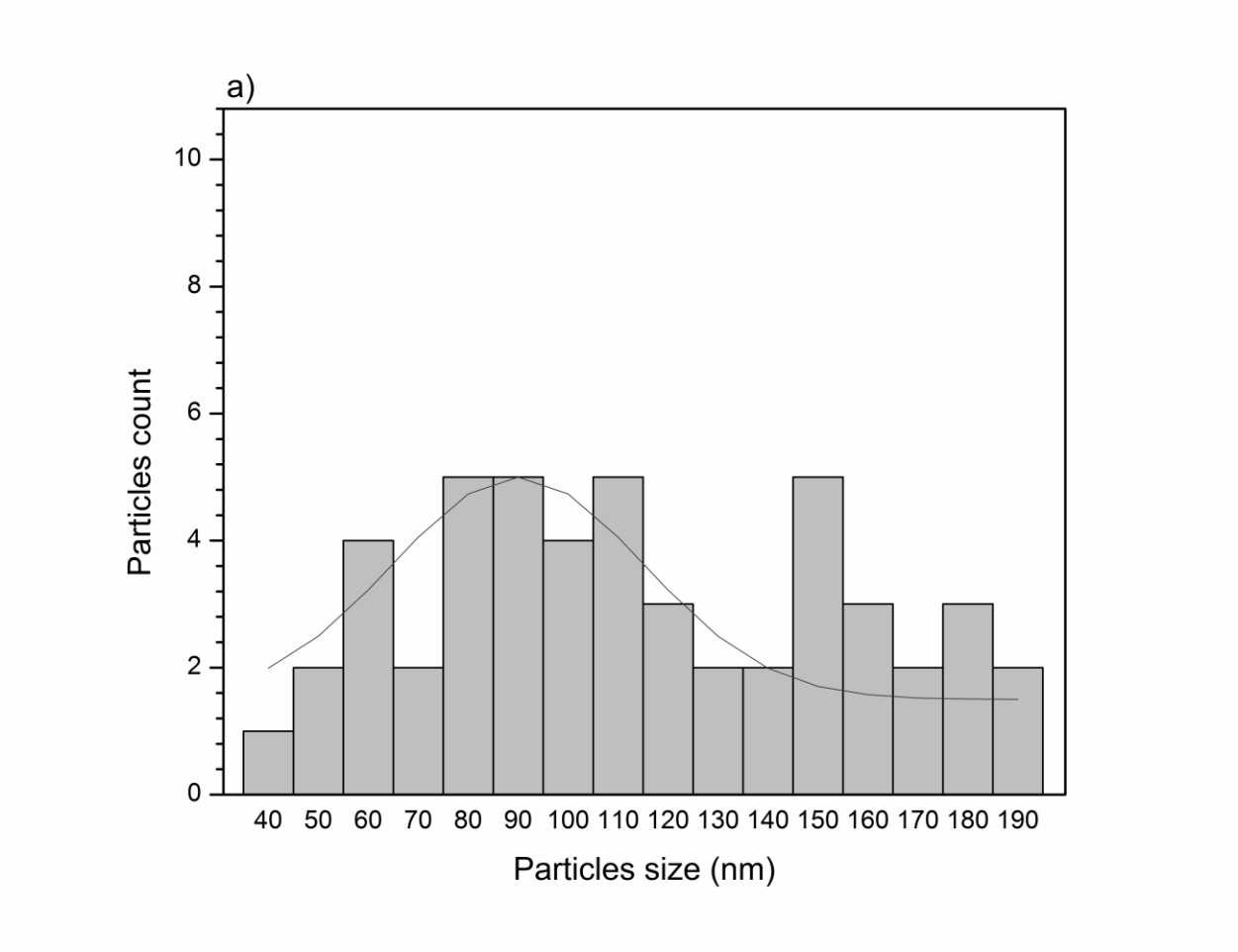

a)
Particles count
0
2
4
6
8
10
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190
Particles size (nm)

Fig. 5b

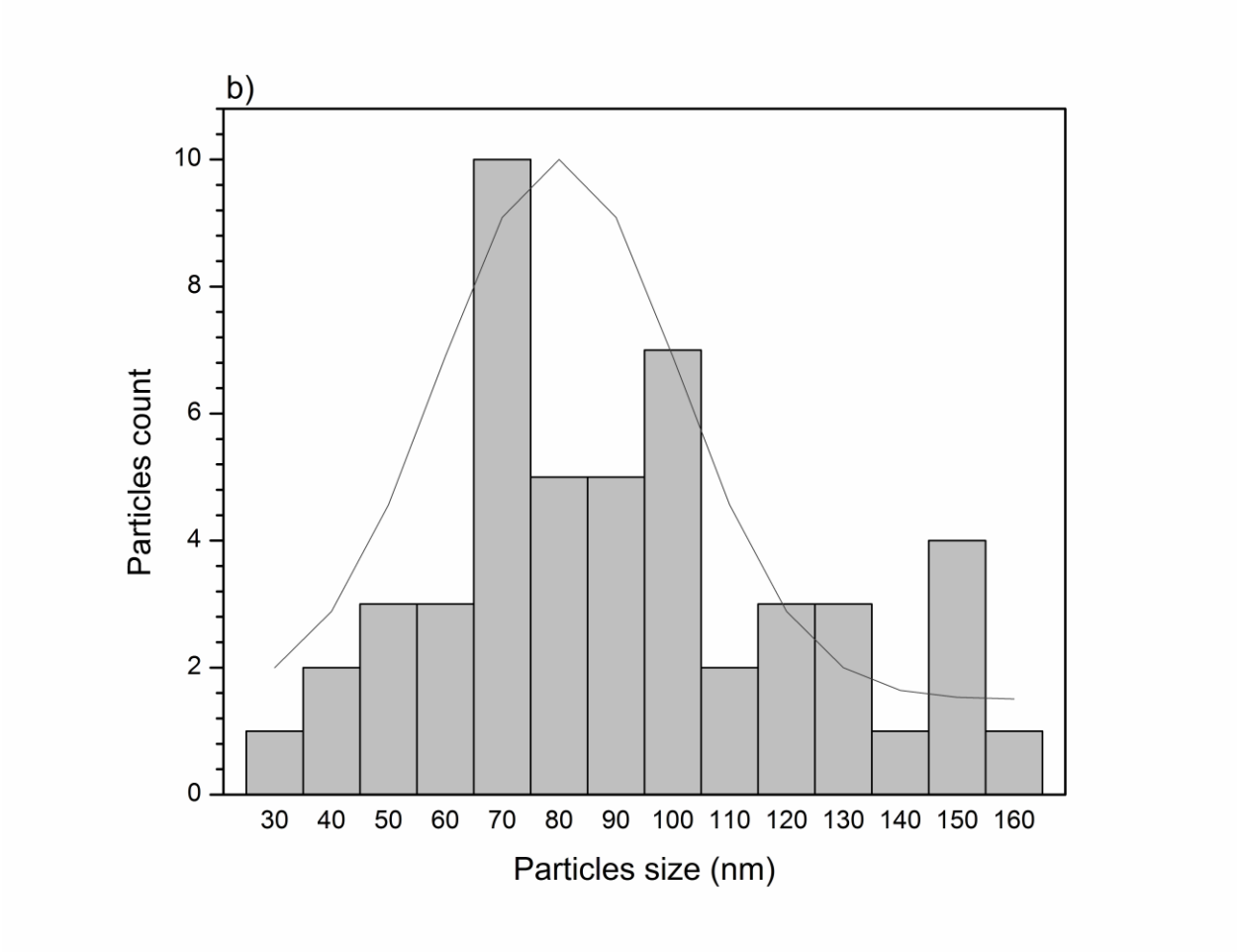

b)
Particles count
0
2
4
6
8
10
30
40
50
60
70
80
90
100
110
120
130
140
150
160
Particles size (nm)

Fig. 6a

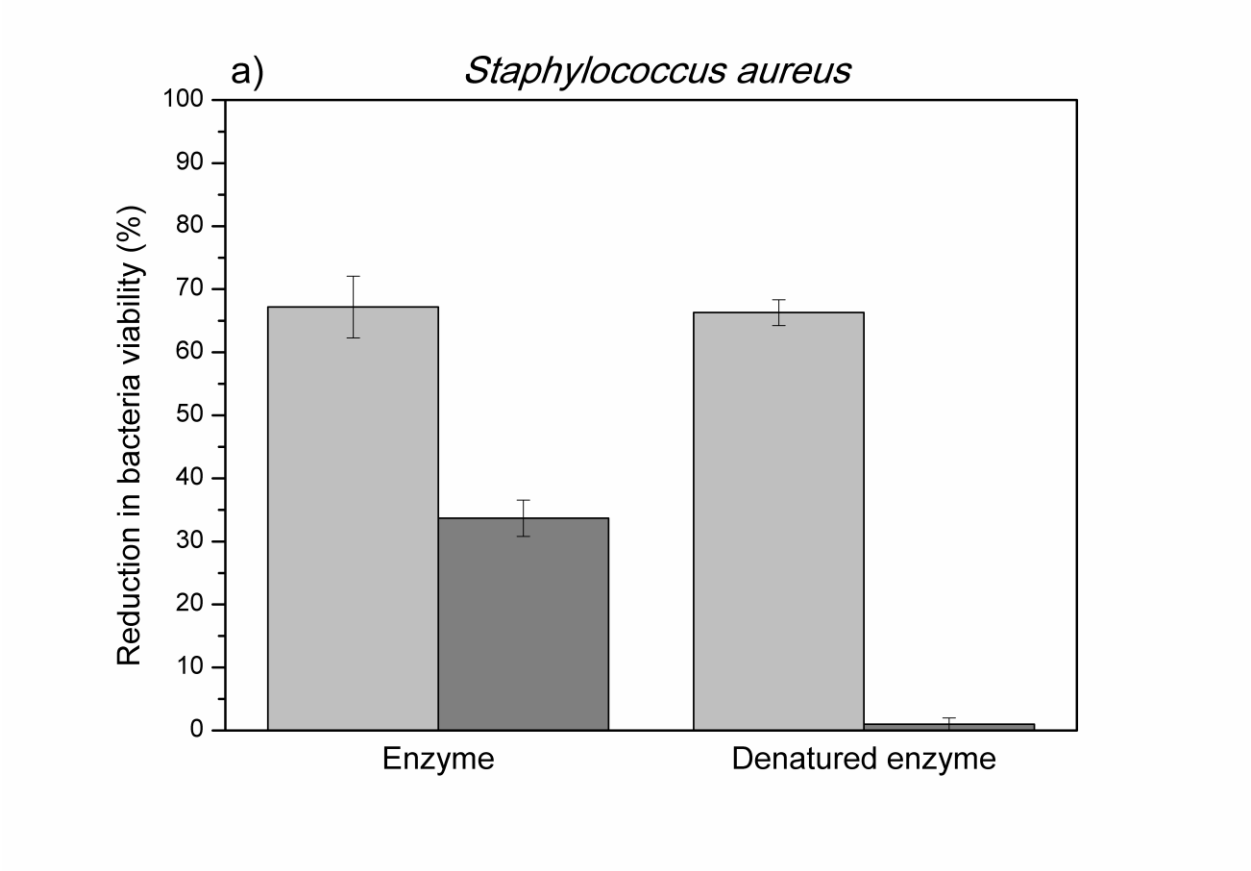
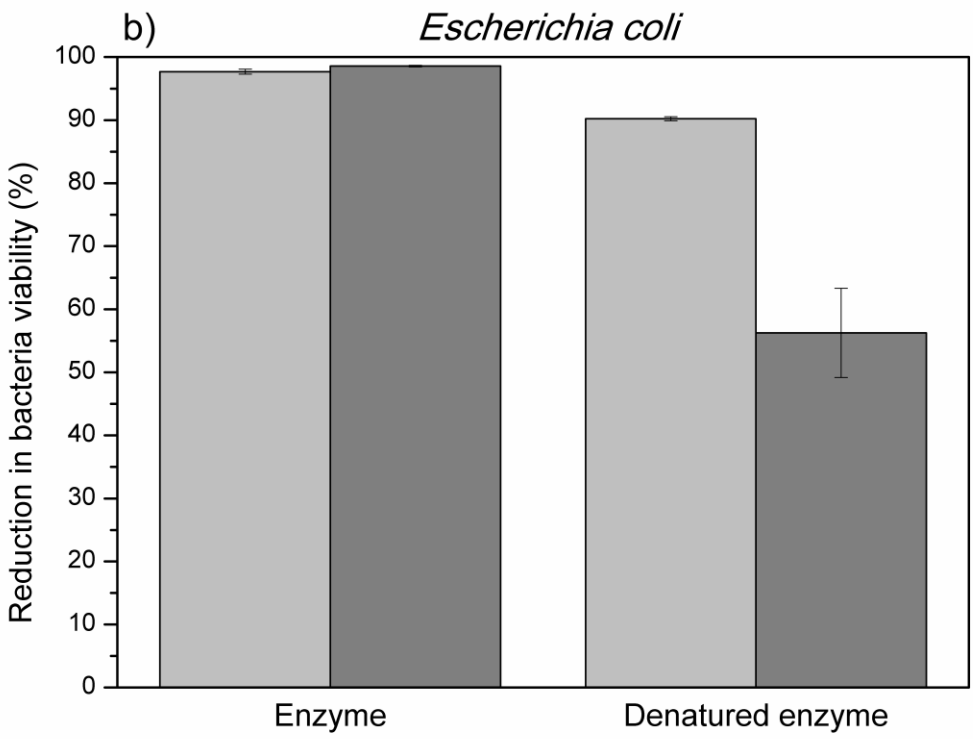

a)
Staphylococcus aureus
Reduction in bacteria viability (%)
100
90
80
70
60
50
40
30
20
10
0
Enzyme
Denatured enzyme

Fig. 6b


b)
Escherichia coli
Reduction in bacteria viability (%)
100
90
80
70
60
50
40
30
20
10
0
Enzyme
Denatured enzyme

Graphical abstract: